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(21) International Application Number: PCT/EP92/02998 (22) International Filing Date: 24 December 1992 (24.12.92) (30) Priority data: 91203408.9 24 December 1991 (24.12.91) EP (34) Countries for which the regional or international application was filed: NL et al. (71) Applicant (for all designated States except US): AKZO N.V. [NL/NL]; Velperweg 76, NL-6824 BM Arnhem (NL). (72) Inventors; and (75) Inventors/Applicants (for US only) : HABETS, Winand, Johannes, Antonius [NL/NL]; 2e Hervendreef 55, NL-5232 JB Den Bosch (NL). HELLINGS, Jan, Albert [NL/NL]; Lierenbout 24, NL-5283 AT Boxtel (NL).		(74) Agent: HERMANS, Franciscus, Guiliemus, Maria; Postbus 20, NL-5340 BH OSS (NL). (81) Designated States: AU, CA, FI, JP, KR, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: PEPTIDES IMMUNOCHEMICALLY REACTIVE WITH ANTIBODIES DIRECTED AGAINST HEPATITIS NON-A, NON-B VIRUS (57) Abstract The invention concerns peptides which react immunochemically with antibodies directed against HCV and nucleic acid sequences encoding these peptides. A method for the detection of HCV or anti-HCV antibodies in a test fluid, an immunochemical reagent and a testkit to be used when applying said detection methods also belong to the invention.		

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Title: Peptides immunochemically reactive with antibodies directed against hepatitis Non-A, Non-B virus.

The invention relates to peptides which react immunochemically with antibodies directed against hepatitis C (HCV) and nucleic acid sequences encoding these peptides.

The invention further relates to a method for the detection of HCV or anti-HCV antibodies in a test fluid, and to an immunochemical reagent and a test kit which may be used in said detection method.

Non-A Non-B hepatitis which is most probably caused by the HCV virus is a transmissible disease or family of diseases shown to be virus-induced. It can be distinguished from other forms of viral-associated liver diseases, including that caused by the known hepatitis viruses, i.e., hepatitis A virus (HAV), hepatitis B virus (HBV), delta hepatitis virus (HDV), and hepatitis induced by cytomegalovirus (CMV) or Epstein-Barr virus (EBV). NANBH was first identified in transfused individuals.

Transmission from man to chimpanzee and serial passage in chimpanzees provided evidence that NANBH is due to a transmissible infectious agent or agents.

Epidemiologic evidence is suggestive that there may be three types of NANBH: the water-borne epidemic type; the blood or needle associated type; and the sporadically occurring (community acquired) type. However, the number of agents which may cause NANBH is unknown.

For the development of a specific and sensitive method to enable a reliable diagnosis to be made in various phases of the infection with NANBH it is of great importance to identify immuno-dominant viral epitopes of this type. The present invention is dealing with new peptides having surprisingly unexpected immunochemical reactivity with anti-HCV antibodies.

The new peptides according to the invention are immunochemically reactive with anti-HCV antibodies, and characterised by the following amino acid sequence (depicted in the one-letter code):

A-X₁-X₂-X₃-X₄-L-X₅-X₆-E-F-X₇-X₈-X₉-B (I),

in which A represents hydrogen, an amino acid or polypeptide; X₁, X₂, X₃, X₄, X₅, X₆, X₇, X₈ and X₉ can be any amino acid; and B represents hydroxy, an amino acid or polypeptide;

or a fragment thereof which is immunologically reactive with anti-HCV antibodies, with the exception of the peptide having the formula A-D-R-E-V-L-Y-R-E-F-D-E-M-B, wherein A and B have the previous defined meaning.

The sequence D-R-E-V-L-Y-R-E-F-D-E-M (II) is part of the HCV sequence and is located in the ORF-region of the SOD/HCV C100-3 clone. A specific immunoreactive peptide comprising the sequence D-R-E-V-L-Y-R-E-F-D-E-M is described in a copending co-owned application with a priority date of 6 December 1991.

It has now been found that, in the above mentioned sequence (II), only three aminoacids are critical with respect to the immune-reactivity with anti-HCV antibodies. Surprisingly, all the other amino acids can be replaced by many other amino acids, while a high reactivity of the peptide towards anti-HCV antibodies is maintained. The present invention therefore provides peptides that can easily be prepared by anyone skilled in the art and have a high immune-reactivity towards anti-HCV antibodies. In the sequence mentioned (I) only the amino acids L, E and F are critical amino acids. These three amino acids can not be replaced by other amino acids without affecting the immune-reactivity with anti-HCV antibodies of the peptides, as can be seen from FIG. 1.

The remaining amino acids X₁-X₉ can be any amino acid with the exception mentioned.

Preferred peptides according to formula I are peptides that differ from the peptide having the formula A-D-R-E-V-L-Y-R-E-F-D-E-M-B in that one amino acid selected from has been replaced by another amino acid.

These peptides have a high immune-reactivity towards anti-HCV antibodies as is shown in figure 1.

In addition to the above peptides, functional derivatives of these peptides are also considered to belong to the peptides according to the instant invention. Functional derivatives of the peptides are meant to include

- a) acid addition salts of the peptides;
- b) amides of the peptides and specifically the C-terminal amides;
- c) esters and specifically C-terminal esters and
- d) N-acyl derivatives, specifically N-terminal acyl derivatives and in particular N-acetyl derivatives.

The peptides according to the invention are particularly suitable for use in a diagnostic method for the determination of the presence of HCV antigens or anti-HCV antibodies in a test fluid.

In contrast to the natural HCV, the peptides according to the invention have the advantage that these are of a safe non-infectious origin. Moreover, the present peptides have a particular high affinity to anti-HCV antibodies, which renders the present peptides extremely suitable for use in the above said diagnostic test methods.

The fragments of the peptides according to formula I are those fragments that differ from fragments of D-R-E-V-L-Y-R-E-F-D-E-M in that one or more amino acids have been replaced by other amino acids.

The preparation of the peptides according to the invention can be effected by means of one of the known organic chemical methods for peptide synthesis or with the aid of recombinant DNA techniques. This latter method involves the preparation of the desired peptide by means of bringing to expression a recombinant nucleic acid sequence with a nucleic acid sequence which is coding for one or more of the peptides in question in a suitable micro-organism as host.

The organic chemical methods for peptide synthesis are considered to include the coupling of the required amino acids by means of a condensation reaction, either in homogeneous phase or with the aid of a so-called solid phase.

The condensation reaction can be carried out as follows:

- a) condensation of a compound (amino acid, peptide) with a free carboxyl group and protected other reactive groups with a compound (amino acid, peptide) with a free amino group and protected other reactive groups where one of the protecting groups also may be a (derivatised) solid support, in the presence of a condensation agent,
- b) condensation of a compound (amino acid, peptide) with an activated carboxyl group and free or protected other reaction groups with a compound (amino acid, peptide) with a free amino group and free or protected other reactive groups, where one of the protecting groups also may be a (derivatised) solid support.

Activation of the carboxyl group can take place, inter alia, by converting the carboxyl group to an acid halide, azide, anhydride, imidazolidine or an activated ester such as the N-hydroxy-succinimide, N-hydroxybenzotriazole, p-nitrophenyl, 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (ODhbt) or pentafluorophenyl (OPfp) ester.

The most common methods for the above condensation reactions are: the carbodiimide method, the BOP method [benzotriazolyl-oxyltris (dimethylamino) phosphonium hexafluoro phosphate] the azide method, the mixed anhydride method and the method using activated esters, such as described in *The Peptides, Analysis, Synthesis, Biology* Vol. 1-3 (Ed. Gross, E. and Meienhofer, J.) 1979, 1980, 1981 (Academic Press, Inc.).

The reactive groups which may not participate in the condensation reaction are, as stated, effectively protected by groups which can be removed again very easily by hydrolysis with the aid of acid, base or reduction. Thus, a carboxyl group can be effectively protected by, for example, esterification with methanol, ethanol, tertiary butanol, benzyl alcohol or p-nitrobenzyl alcohol and amidation with amines or derivatives of alcohols and amines linked to solid supports.

Groups which can effectively protect an amino group are the ethoxycarbonyl, benzyloxycarbonyl, t-butoxy-carbonyl,

9-fluorenyl-methoxycarbonyl(Fmoc) or p-methoxy-benzyloxycarbonyl group, or an acid group derived from a sulphonic acid, such as the p-toluene-sulphonyl, penta-methylbenzene sulfonyl (Pms), 4-methoxy-2,3,6-trimethyl-benzene sulfonyl (Mtr) or 1,2,5,7,8-pentamethylchroman-6-sulfonyl (Pmc) group, but other groups can also be used, such as substituted or unsubstituted aryl or aralkyl groups, for example benzyl and triphenylmethyl, or groups such as ortho-nitrophenyl-sulphenyl and 2-benzoyl-1-methylvinyl.

A more extensive account of possible protecting groups can be found in The peptides, Analysis, Synthesis, Biology Vol. 1-9 (Eds. Gross, Udenfriend and Meienhofer) 1979-1987 (Academic Press, Inc.).

Preparation of the abovementioned peptides according to the invention using the "solid phase" method, is for example described in J.Am.Chem.Soc., 85, 2149 (1963) and Int.J.Peptide Protein Res., 35, 161-214 (1990). The coupling of the amino acids of the peptide to be prepared usually starts from the carboxyl end side. For this method a solid phase is needed on which there are reactive groups or on which such groups can be introduced. This can be, for example, a copolymer of benzene and divinylbenzene with reactive chloromethyl groups, or a polymeric solid phase rendered reactive with hydroxymethyl or amine-functions.

A particularly suitable solid phase is, for example, the p-alkoxybenzyl alcohol resin (4-hydroxymethyl-phenoxy-methyl-copolystyrene-1% divinylbenzene resin), described by Wang (1974) J.Am.Chem.Soc., 95, 1328. After synthesis the peptides can be split from this solid phase under mild conditions. Other suitable supports are derivatised polyethylene or polypropylene rods as described by Geysen, Proc. Natl. Acad. Sci., 81, 3998 (1984) and Proc. Natl. Acad. Sci., 82, 178 (1985).

After synthesis of the desired amino acid sequence, either in solution or on a solid support, the protective groups can be split off by various conventional methods, depending on the nature of the particular group, for example with the aid of trifluoro-acetic acid or by mild

reduction, for example with hydrogen and a catalyst, such as palladium, treatment with a base as for example piperidine or hydroxide ions, or with HBr in glacial acetic acid.

If the peptide is synthesized on a solid support from which it can be detached this can be achieved, depending on the type of linker, for example, with trifluoro-acetic acid, trifluoromethanesulphonic acid or with methanesulphonic acid dissolved in trifluoro-acetic acid, transesterification with a lower alcohol, preferably methanol or ethanol, in which case a lower alkyl ester of the peptide is formed directly. Likewise, splitting with the aid of ammonia gives the amide of a peptide according to the invention.

As already indicated above, the peptide according to the invention can likewise be prepared with the aid of recombinant DNA techniques. This possibility is of importance particularly when the peptide is incorporated in a repeating sequence ("in tandem") or when the peptide can be prepared as an essential constituent of a (much larger) protein or polypeptide. This type of preparation of the peptide therefore likewise falls within the scope of the invention. For this purpose, as a constituent of a recombinant DNA, a nucleic acid sequence is used which codes for the peptide according to the invention and which, furthermore, is substantially free from polynucleotide segments, which in the naturally occurring HCV genome flank the nucleic acid sequence indicated above.

A nucleic acid sequence of this type, which is coding for the peptide according to the invention, and a recombinant DNA in which this nucleic acid sequence is incorporated likewise fall within the scope of the invention.

"Nucleic acid sequence" as used herein refers to a polymeric form of nucleotides of any length, both to ribonucleic acid sequences and to deoxy ribonucleic acid sequences. In principle, this term refers to the primary structure of the molecule. Thus, this term includes double

and single stranded DNA, as well as double and single stranded RNA, and modifications thereof.

A nucleic acid sequence according to the present invention can be ligated to various replication effecting DNA sequences with which it is not associated or linked in nature resulting in a so called recombinant vector molecule which can be used for the transformation of a suitable host. Useful recombinant vector molecules, are preferably derived from, for example plasmids, bacteriophages, cosmids or viruses.

The methods to be used for the construction of a recombinant vector molecule according to the invention are known to those of ordinarily skill in the art and are inter alia set forth in Maniatis, T. et al. (Molecular Cloning A Laboratory Manual, second edition; Cold Spring Harbor Laboratory, 1989).

For example, the insertion of the nucleic acid sequence according to the invention into a cloning vector can easily be achieved when both the genes and the desired cloning vehicle have been cut with the same restriction enzyme(s) as complementary DNA termini are thereby produced.

For expression nucleic acid sequences of the present invention in an expression vector, said sequences are operably linked to expression control sequences. Such control sequences may comprise promoters, enhancers, operators, inducers, ribosome binding sites etc.

It should, of course, be understood that the nucleotide sequences inserted at the selected site of the cloning vector may include only a fragment of the complete nucleic acid sequence encoding for the peptides according to the invention as long as the transformed host will produce a polypeptide having at least one or more immunogenic determinants .

The recombinant vector molecules may additionally contain one or more marker activities that may be used to select for desired transformants, such as ampicillin and tetracycline resistance in pBR322, as for example ampicillin resistance and α -peptide of β -galactosidase in pUC8.

The peptides or fragments thereof prepared and described above are used to produce antibodies, both polyclonal and monoclonal. Monoclonal antibodies directed against peptides according to the invention can be readily produced by one skilled in the art.

Making monoclonals by hybridomas is well known. Cell fusion, immortal antibody-producing cell lines can be created while also other techniques are available such as direct transformation of B-lymphocytes with oncogenic DNA or transfection with Epstein-Barr Virus.

Antibodies, both monoclonal and polyclonal, directed against peptides according to the invention are very suitable in diagnosis, while those antibodies which are neutralizing are very useful in passive immunotherapy. Especially monoclonal antibodies may be used to raise anti-idiotypic antibodies. Techniques for raising anti-idiotypic antibodies are known in the art.

Said anti-idiotypic antibodies are also useful for prevention and/or treatment of Non-A, Non-B Hepatitis, as well as for the elucidation of important epitopic regions of HCV-antigens.

The present invention further comprises an immunochemical reagent which reagent contains one or more of the peptides of the invention.

The "immunochemical reagent" according to the invention usually comprises one or more peptides according to the invention and a suitable support or a labelling substance. A support that may be used in this respect is, for example, a carrier protein such as BSA, the inner wall of a microtest well or a cuvette, a tube or capillary, a membrane, filter, test strip or a particle such as, for example, a latex particle, an erythrocyte, a dye sol, a metal sol or metal compound as sol particle.

A labelling substance that may be used can be a radioactive isotope, a fluorescent compound, a labelling protein such as (an enzyme), an enzyme, a dye sol, metal sol or metal compound as sol particle.

The said immunochemical reagent is the essential component in a method for the detection of antibodies directed against HCV in a test fluid. The immunochemical reagent is therefore brought into contact with the said test fluid

whereby an immunochemical reaction takes place resulting in the formulation of an immune complex between the peptide (according to the invention) and the anti-HCV antibodies. Depending on the nature and further characteristics of the immunochemical reagent the immunochemical reaction that takes place is a so-called sandwich reaction, an agglutination reaction, a competition reaction or an inhibition reaction. The immune complex formed as a result of the above reaction is a (direct or indirect) measure for the presence of antibodies in the test fluid.

The immunochemical reagent according to the invention can also be used for the detection of HCV antigen in a test fluid.

The invention further comprises a method for the detection of antibodies directed against HCV in a test fluid, whereby one or more of the peptides according to the invention are used.

The invention also relates to a method for the detection of HCV in a test fluid, using one or more of the peptides according to the invention.

In this detection method the test fluid can be contacted with anti-HCV and subsequently or simultaneously with the immunochemical reagent according to the invention.

A particularly suitable method for the detection of HCV in a test fluid is based on a competition reaction between a peptide according to the invention provided with a labelling substance and a HCV antigen (present in the test fluid), whereby the peptide and the antigen are competing with the antibody directed against HCV attached to a solid support.

The invention further comprises a test kit to be used for carrying out an immuno-assay, this test kit containing at least one immunochemical reagent according to the invention. A test kit according to the invention comprises, as an essential constituent, an immunochemical reagent as described above. In carrying out a sandwich reaction for the detection of anti-HCV antibodies the test kit may comprise, for example,

1) the peptide according to the invention attached to a solid support, (for example the inner wall of a microtest well) and

2) either a labelled peptide according to the invention or a labelled anti-antibody.

In carrying out a competition reaction for detection HCV antibodies, the test kit may comprise

1) the peptide according to the invention attached to a solid support, and

2) a labelled antibody directed against HCV preferably a monoclonal antibody directed against said peptide.

In carrying out an agglutination reaction the test kit comprises an immunochemical reagent which consists of a peptide according to the invention attached to particles or sols.

A test kit for the detection of HCV antigen comprises, for example, a labelled peptide according to the invention and an antibody directed against HCV, which is attached to a solid support.

The invention is further exemplified by the following example.

Example:

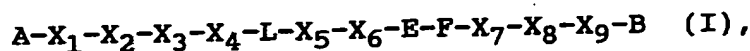
Peptides were synthesized which differed from the peptide having the formula D-R-E-V-L-Y-R-E-F-D-E-M, in that one amino acid was replaced by another aminoacid. Peptides were synthesized in which each amino acid of the above mentioned sequence was replaced by any aminoacid selected from the group; A, C, D, E, F, G, H, I, K, L, M, N, P, Q, R, S, T, V, W and Y. The resulting peptides were all tested for its immune reactivity with human and mouse anti-HCV sera. The results of this procedure are shown in figure 1. In this figure two graphs are presented (one showing immune-reactivity with human anti-HCV sera and one showing the immune-reactivity with mouse anti HCV sera), for each amino acid from the above mentioned sequence. These graphs show the immune-reactivity of all peptides in which the original aminoacid (either D, R, E, V, L, Y, R, E, F, D, E or M) has been replaced by any other amino acid from the above mentioned group. The bold line in each graph represents the immuno-reactivity of the peptide with formula D-R-E-V-L-Y-R-E-F-D-E-M. From this figure it can

be seen that most of the amino acids can easily be replaced by any other aminoacid without affecting the immune-reactivity. Only the amino acids L, E and F (as depicted in formula I)) can not be replaced by any other amino acid without negatively affecting the immune-reactivity.

Figure 1: Immune reactivity, with human and mouse anti-HCV sera, of peptides that differ from the peptide with formula D-R-E-V-L-Y-R-E-F-D-E-M in that one amino acid has been replaced by any amino acid selected from the group A, C, D, E, F, G, H, J, K, L, M, N, P, Q, R, S, T, V, W and Y.

Claims

1. A peptide with amino acid sequence



or a fragment thereof,

in which A represents hydrogen, an amino acid or a polypeptide; X_1 , X_2 , X_3 , X_4 , X_5 , X_6 , X_7 , X_8 and X_9 can be any amino acid; and B represents hydroxy, an amino acid or polypeptide; being immunochemically reactive with anti-HCV antibodies, with the exception of the peptide having the formula A-D-R-E-V-L-Y-R-E-F-D-E-M-B, wherein A and B have the previous defined meaning.

2. A peptide according to claim 1, that differs from the peptide having the formula A-D-R-E-V-L-Y-R-E-F-D-E-M-B in that one amino acid has been replaced by a different amino acid.
3. Nucleic acid sequence encoding a peptide according to claim 1 or 2.
4. Immunochemical reagent which contains a peptide according to Claim 1 or 2.
5. Method for the detection of antibodies directed against HCV in a test fluid, characterized in that an immunochemical reagent according to Claim 2 is brought into contact with the test fluid and the presence of immune complexes formed between the peptide and antibodies in the test fluid, is detected, which is a measure for the presence of anti-HCV antibodies in the test fluid.
6. Method for the detection of HCV in a test fluid, characterized in that an immunochemical reagent according to Claim 2 is brought into contact with the test fluid and anti-HCV antibodies, whereafter the presence of immune complexes formed is detected and from this the presence of HCV in the test fluid is determined.

7. Test kit for carrying out the method according to claim 5 or 6.

FIGURE 1

Replacement net of the MoAb 8N epitope analyzed with
a human anti HCV serum and MoAb 8N

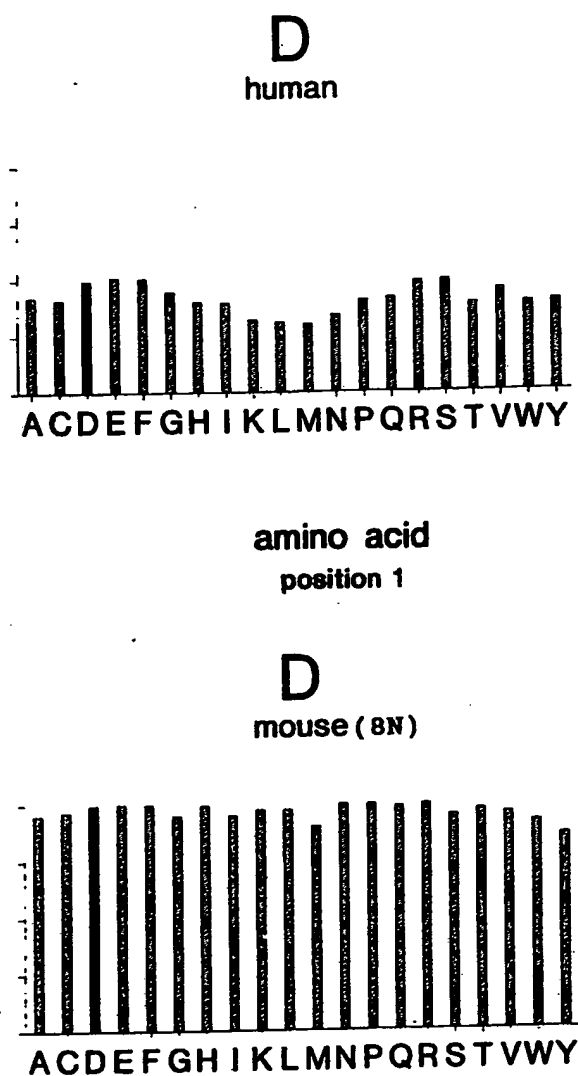
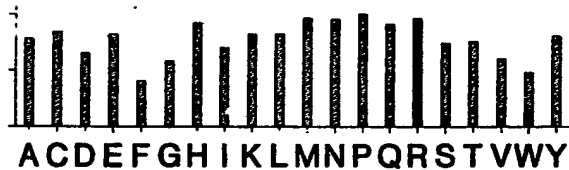


FIGURE 1 (continued)

R
human



amino acid
position 2

R
mouse (8N)

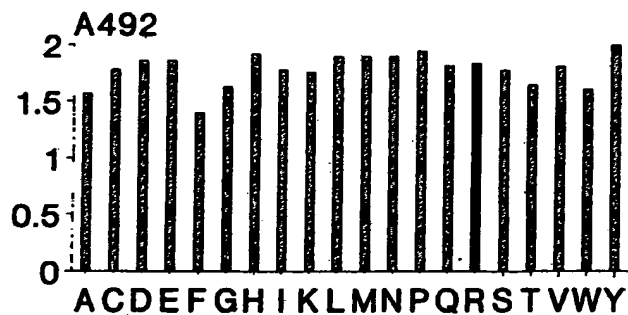
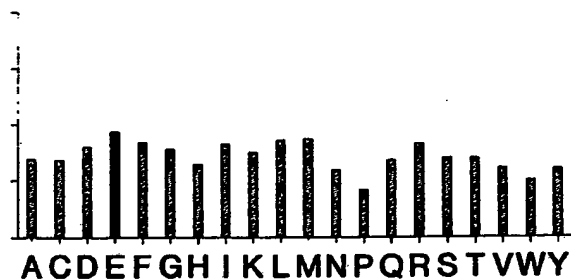


FIGURE 1 (continued)

E
human



amino acid
position 3

E
mouse (8N)

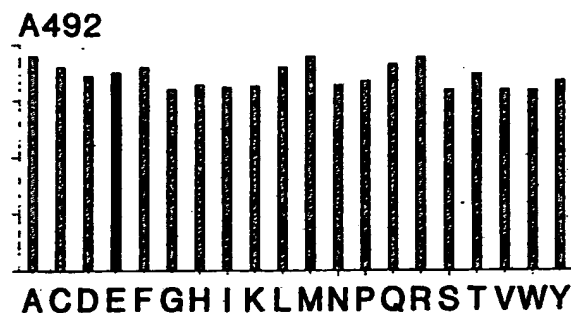
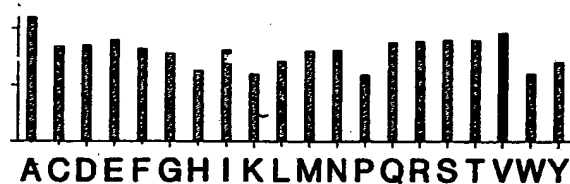


FIGURE 1 (continued)

V
human



amino acid
position 4

V
mouse (8N)

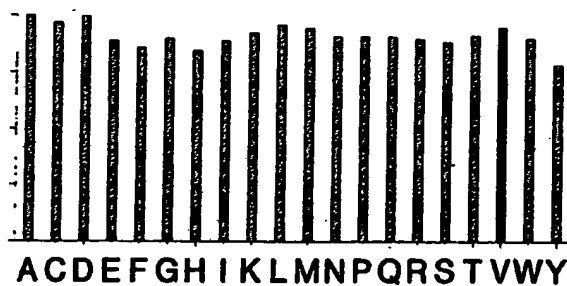
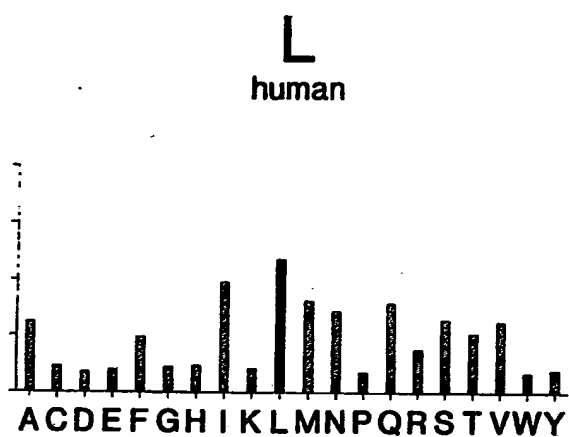


FIGURE 1 (continued)



amino acid
position 5

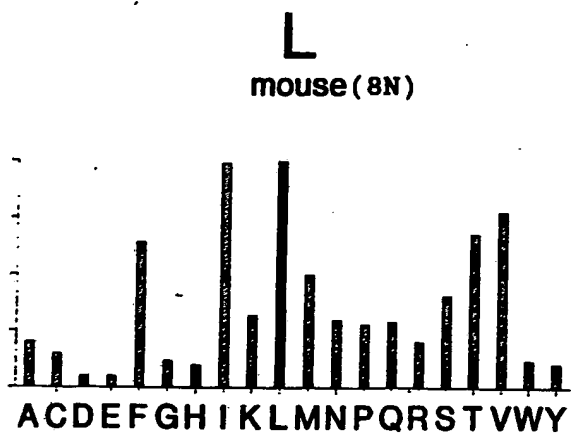
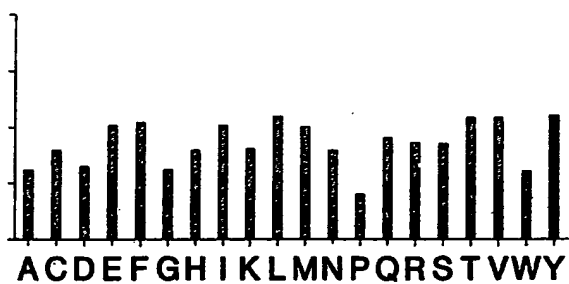


FIGURE 1 (continued)

Y
human



amino acid
position 6

Y
mouse (8N)

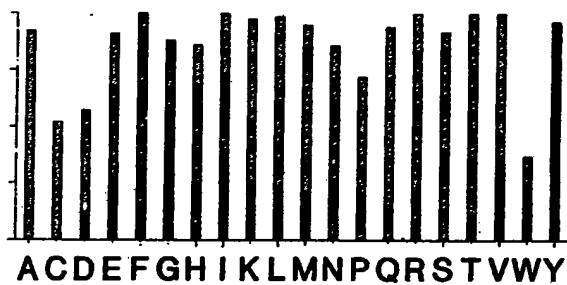
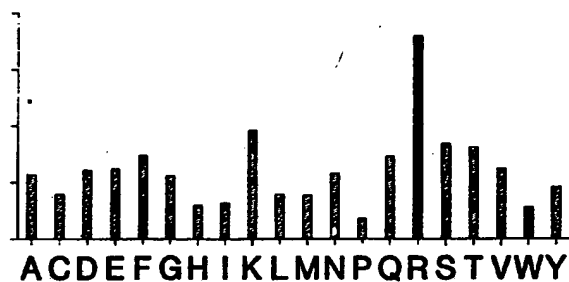


FIGURE 1 (continued)

R
human



amino acid
position 7

R
mouse (8N)

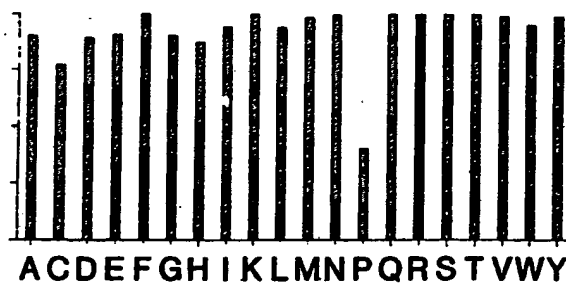
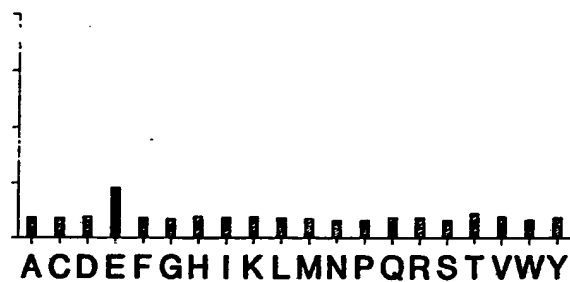


FIGURE 1 (continued)

E
human



amino acid
position 8

E
mouse (8N)

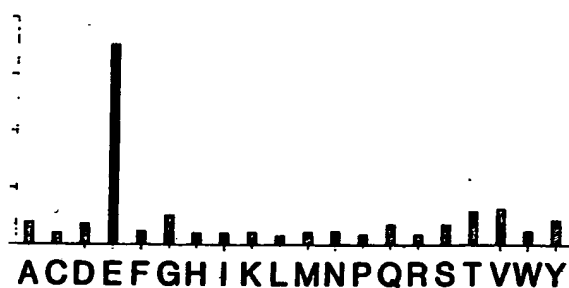
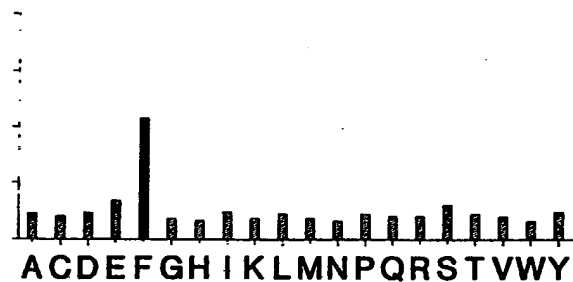


FIGURE 1 (continued)

F
human



amino acid
position 9

F
mouse (8N)

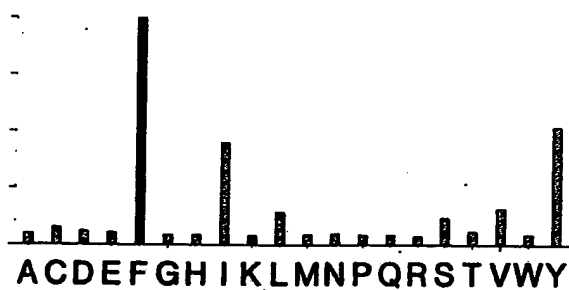
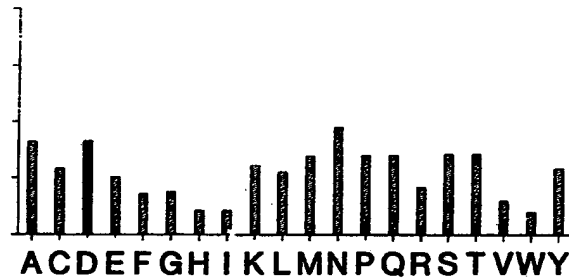


FIGURE 1 (continued)

D
human



amino acid
position 10

D
mouse (8N)

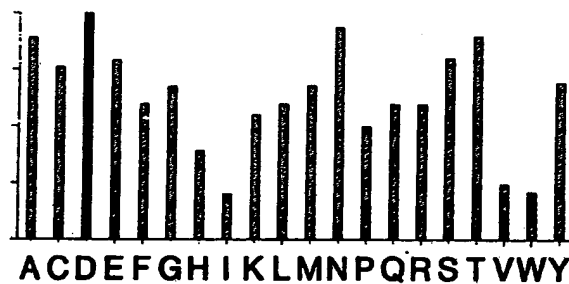
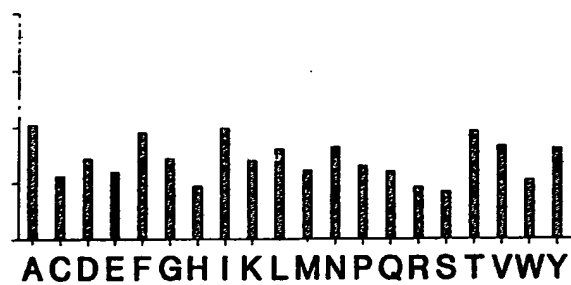


FIGURE 1 (continued)

E
human



amino acid
position 11

E
mouse (8N)

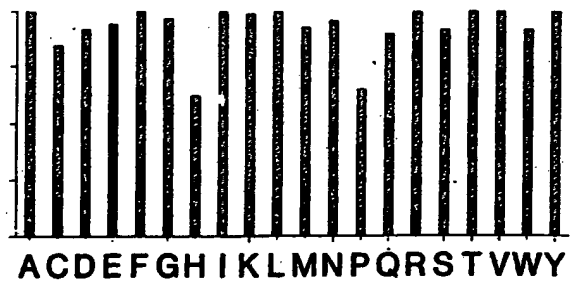
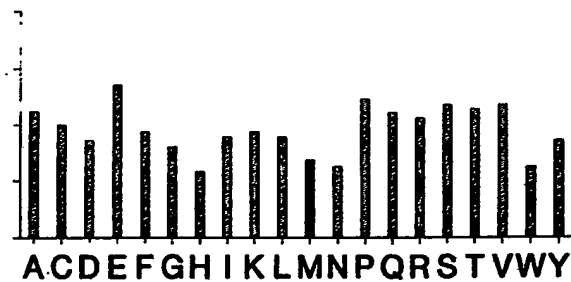


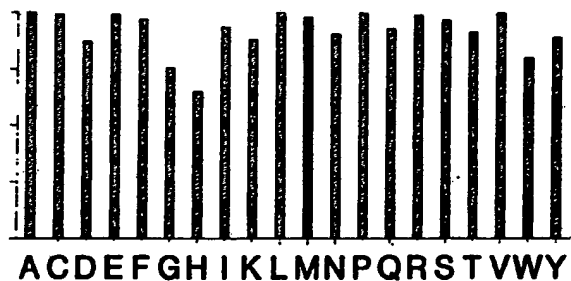
FIGURE 1 (continued)

M
human



amino acid
position 12

M
mouse (8N)



INTERNATIONAL SEARCH REPORT

PCT/EP 92/02998

International Application No

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5	C07K7/08; A61K39/29	C07K7/10; G01N33/576; G01N33/68
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	C07K ; G01N	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	DE,A,3 707 710 (HOECHST A.-G.) 22 September 1988 * See formula I *	1
X	EP,A,0 442 394 (UNITED BIOMEDICAL, INC.) 21 August 1991 * See the whole document, especially page 42, line 50 *	1,4-7
P,X	WO,A,9 210 514 (INNOGENETICS N.V.) 25 June 1992 * See the whole document, especially page 4, line 17-21 and page 7, peptides VIII & IX *	1,4-7
	--- -/--	
<p>¹⁰ Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search		Date of Mailing of this International Search Report
04 MARCH 1993		13. 03. 93
International Searching Authority EUROPEAN PATENT OFFICE		Signature of Authorized Officer KORSNER S.E.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	EP,A,0 463 848 (THE RESEARCH FOUNDATION FOR MICROBIAL DISEASES OF OSAKA UNIVERSITY) 2 January 1992 * See page 46, lines 44-48 and page 59, line 50 * -----	1,3-4

**ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.**

EP 9202998
SA 68653

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

04/03/93

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WO-A-9210514	25-06-92	EP-A- 0489968 AU-A- 9068991	17-06-92 08-07-92
EP-A-0463848	02-01-92	AU-A- 7925691 CA-A- 2045326 CN-A- 1059758 AU-A- 6860891 CA-A- 2045323 CN-A- 1057861 EP-A- 0464287	02-01-92 26-12-91 25-03-92 02-01-92 26-12-91 15-01-92 08-01-92